L2: Entry 1 of 98 File: PGPB Jul 5, 2001

DOCUMENT-IDENTIFIER: US 20010006802 A1
TITLE: METHODS FOR SEPARATION AND PURIFICATION OF BIOPOLYMERS

BSTX:
[0003] Polyhydroxyalkanoates (PHAs) are thermoplastic polyesters which can be produced from bacteria or plants (Williams & Peoples, CHEMTECH 26:33-44 (1996)). These polymers can be recovered from the biological systems (the biomass) by organic solvent processes, aqueous processes, or a combination of both organic solvent/aqueous processing. Examples of known organic solvent recovery processes are described in U.S. Pat. No. 4,310,684 and U.S. Pat. No. 4,705,604 to Vanlautem et al. (extraction of PHB from microbes with chlorinated solvents); U.S. Pat. No. 4,968,611 to Traussnig et al. (use of diols, acetalized triols, di- or tricarboxylic acid esters or butyrolactone to extract poly-3-hydroxybutyrate (PHB) and its copolymers from microbes); U.S. Pat. No. 5,213,976 to Blauhut et al. (process for extracting PHB from microbial cells using methylene chloride followed by precipitation of the PHB in water); PCT WO 97/15681; PCT WO 93/11656 (use of acetone to extract poly-3-hydroxyoctanoate polymer from Pseudomonas oleovorans); PCT WO 96/06179 and PCT WO 97/15681 (solvent methods for recovering PHAs from transgenic plant crops); and U.S. Pat. No. 5,821,299 to Noda (the use of solvent/partial non-solvent mixtures for extracting PHAs from biomass). Typically, in each of these prior art processes, some of the biomass components are co-extracted with the PHA, which can cause the PHA product to be discolored and/or to have an unpleasant odor.

BSTX:
[0011] Methods are provided for the recovery and purification of polyhydroxyalkanoates (PHAs) from PHA-containing plant and microbial biomass, wherein the methods include contacting the biomass or partially purified PHA with ozone in at least one step of a purification process. Ozone has the beneficial effects of (a) bleaching, (b) deodorization, and (c) solubilization of impurities, facilitating their removal from aqueous polymer suspensions or latexes. The ozone treatment may be used alone or in combination with other treatment, extraction, and separation processes, and is especially suitable for the treatment of PHA-containing latexes, slurries, suspensions, and organic solvent solutions. The ozone contacting step advantageously can be conducted over a wide range of temperatures, including processing temperatures, for example between about 1 and 40.degree. C., which are lower than processing temperatures used in known methods. Treatment with ozone of PHA-containing biomass, partially purified PHA, or solvent-extracted PHA yields an enhanced level of polymer purity suitable for coating and other applications. The ozone treatment also has the added advantage that the resulting PHA polymer or polymer latex is essentially odor-free.

BSTX:
[0014] PHAs can be produced in a number of biological systems including bacteria and genetically engineered plant crops. In bacterial systems, the PHAs are accumulated intracellularly as granular inclusion bodies. PHA also can be produced in genetically engineered plant crops. Methods for constructing such crops are described, for example, in U.S. Pat. Nos. 5,245,023 and 5,250,430 to Peoples and Sinskey; U.S. Pat. No. 5,502,273 to Bright et al.; U.S. Pat. No. 5,534,432 to Peoples and Sinskey; U.S. Pat. No. 5,602,321 to John; U.S. Pat. No. 5,610,041 to Somerville et al.; PCT WO 91/00917; PCT WO 92/19747; PCT WO 93/02187; PCT WO 93/02194; PCT WO 94/12014, Poirier et al., Science 256:520-23 (1992); van der Leij & Witholt, Can. J Microbiol. 41(supp.):222-38 (1995); Nawrath & Poirier, The International Symposium on Bacterial Polyhydroxyalkanoates, (Eggink et al., eds.) Davos Switzerland (Aug. 18-23, 1996); and Williams & Peoples, CHEMTECH 26: 38-44 (1996). Methods for recovering

1996); and Williams & Peoples, CHEMTECH 26: 38-44 (1996). Methods for recovering PHAs from plant biomass are described, for example in PCT WO 97/15681, PCT WO 97/07239, and PCT WO 97/07229.

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☐ 1. Document ID: US 20010006802 A1

L2: Entry 1 of 98 File: PGPB Jul 5, 2001

PGPUB-DOCUMENT-NUMBER: 20010006802
PGPUB-FILING-TYPE: new-utility
DOCUMENT-IDENTIFIER: US 20010006802 A1

TITLE: METHODS FOR SEPARATION AND PURIFICATION OF BIOPOLYMERS

PUBLICATION-DATE: July 5, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
HOROWITZ, DANIEL M.	SOMERVILLE	MA	US	
BRENNAN, ELAINE M.	SOMERVILLE	MA	US	

US-CL-CURRENT: 435/135; 435/132

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 6265202 B1

L2: Entry 2 of 98 File: USPT Jul 24, 2001

US-PAT-NO: 6265202
DOCUMENT-IDENTIFIER: US 6265202 B1

TITLE: DNA encoding methymycin and pikromycin

DATE-ISSUED: July 24, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sherman; David H.	St. Louis Park	MN	N/A	N/A
Liu; Hung-Wen	Roseville	MN	N/A	N/A
Xue; Yongquan	St. Paul	MN	N/A	N/A
Zhao; Lishan	St. Paul	MN	N/A	N/A

US-CL-CURRENT: 435/252.31; 435/183, 435/252.3, 435/252.33, 435/320.1, 536/23.1, 536/23.2, 536/23.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 3. Document ID: US 6245537 B1

L2: Entry 3 of 98 File: USPT Jun 12, 2001

US-PAT-NO: 6245537
DOCUMENT-IDENTIFIER: US 6245537 B1

TITLE: Removing endotoxin with an oxdizing agent from polyhydroxyalkanoates produced by fermentation

DATE-ISSUED: June 12, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Williams; Simon F.	Sherborn	MA	N/A	N/A
Martin; David P.	Arlington	MA	N/A	N/A
Gerngross; Tillman	Cambridge	MA	N/A	N/A
Horowitz; Daniel M.	Somerville	MA	N/A	N/A

US-CL-CURRENT: 435/135; 424/422, 424/486, 435/170, 435/180, 528/271

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw. Desc | Image

☐ 4. Document ID: US 6228934 B1

L2: Entry 4 of 98 File: USPT May 8, 2001

US-PAT-NO: 6228934
DOCUMENT-IDENTIFIER: US 6228934 B1

TITLE: Methods and apparatus for the production of amorphous polymer suspensions

DATE-ISSUED: May 8, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Horowitz; Daniel	Somerville	MA	N/A	N/A
Gerngross; Tillman U.	Hanover	NH	N/A	N/A

US-CL-CURRENT: 524/800; 524/801, 524/802, 525/437, 525/450, 526/62, 528/499, 528/503

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw. Desc | Image

☐ 5. Document ID: US 6228623 B1

L2: Entry 5 of 98 File: USPT May 8, 2001

US-PAT-NO: 6228623
DOCUMENT-IDENTIFIER: US 6228623 B1

TITLE: Polyhydroxyalkanoates of narrow molecular weight distribution prepared in transgenic plants

DATE-ISSUED: May 8, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Asrar; Jawed	Chesterfield	MO	N/A	N/A
Mitsky; Timothy A.	Maryland Heights	MO	N/A	N/A
Shah; Devang T.	Chesterfield	MO	N/A	N/A

US-CL-CURRENT: 435/135; 435/155, 435/157, 435/158, 528/1, 530/200

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

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☐ 6. Document ID: US 6207217 B1

L2: Entry 6 of 98 File: USPT Mar 27, 2001

US-PAT-NO: 6207217
DOCUMENT-IDENTIFIER: US 6207217 B1

TITLE: Animal nutrition compositions

DATE-ISSUED: March 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Peoples; Oliver P.	Arlington	MA	N/A	N/A
Saunders; Court	Clive	IA	N/A	N/A
Nichols; Scott	Johnston	IA	N/A	N/A
Beach; Larry	Des Moines	IA	N/A	N/A

US-CL-CURRENT: 426/635; 426/49, 426/623, 426/630, 426/807

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

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☐ 7. Document ID: US 6201083 B1

L2: Entry 7 of 98 File: USPT Mar 13, 2001

US-PAT-NO: 6201083
DOCUMENT-IDENTIFIER: US 6201083 B1

TITLE: Modified polyhydroxyalkanoates for production of coatings and films

DATE-ISSUED: March 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Asrar; Jawed	Chesterfield	MO	N/A	N/A
D'Haene; Pol	Kessel-Lo	N/A	N/A	BEX

US-CL-CURRENT: 526/238.3; 528/295.5, 528/300, 528/308, 560/127

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

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☐ 8. Document ID: US 6194008 B1

L2: Entry 8 of 98 File: USPT Feb 27, 2001

US-PAT-NO: 6194008
DOCUMENT-IDENTIFIER: US 6194008 B1

TITLE: Environmentally friendly chewing gum bases including polyhydroxyalkanoates

DATE-ISSUED: February 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Li; Weisheng	Montclair	NJ	N/A	N/A
Orfan; Charles P.	Howell	NJ	N/A	N/A
Liu; Jingping	Highland Park	NJ	N/A	N/A
Foster; John W.	Piscataway	NJ	N/A	N/A

US-CL-CURRENT: 426/6

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

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☐ 9. Document ID: US 6191203 B1

L2: Entry 9 of 98 File: USPT Feb 20, 2001

US-PAT-NO: 6191203
DOCUMENT-IDENTIFIER: US 6191203 B1

TITLE: Polymer blends containing polyhydroxyalkanoates and compositions with good retention of elongation

DATE-ISSUED: February 20, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Asrar; Jawed	Chesterfield	MO	N/A	N/A
Pierre; Jean R.	St-Denis	N/A	N/A	BEX

US-CL-CURRENT: 524/317; 524/308, 525/437, 525/444, 525/450, 528/354, 528/361

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

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☐ 10. Document ID: US 6175061 B1

L2: Entry 10 of 98 File: USPT Jan 16, 2001

US-PAT-NO: 6175061
DOCUMENT-IDENTIFIER: US 6175061 B1

TITLE: Production of polyhydroxyalkanoate in plants

DATE-ISSUED: January 16, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bright; Simon William Jonathan	Marlow	N/A	N/A	GBX
Byrom; David	Cleveland	N/A	N/A	GBX
Fentem; Philip Anthony	Berkshire	N/A	N/A	GBX

US-CL-CURRENT: 800/298; 800/306, 800/312, 800/317.3, 800/322

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

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PLANTAE.DWPI,EPAB,JPAB,USPT,PGPB.	38
PLANTAG.DWPI,EPAB,JPAB,USPT,PGPB.	2
PLANTAL.DWPI,EPAB,JPAB,USPT,PGPB.	5
PLANTAN.DWPI,EPAB,JPAB,USPT,PGPB.	127
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L2: Entry 2 of 98 File: USPT Jul 24, 2001

DOCUMENT-IDENTIFIER: US 6265202 B1
TITLE: DNA encoding methymycin and pikromycin

BSPR:
The present invention provides a method of preparing a polyhydroxyalkanoate synthase. The method comprises introducing an expression cassette into a non-plant eukaryotic cell. The expression cassette comprises a DNA molecule encoding a polyhydroxyalkanoate synthase, e.g., a polyhydroxybutyrate synthase, operably linked to a promoter functional in the non-plant eukaryotic cell. The DNA molecule may be obtained from a bacterium such as Alcaligenes eutrophus. The DNA molecule encoding the polyhydroxyalkanoate synthase is then expressed in the cell. Thus, another embodiment of the invention provides a purified recombinant polyhydroxybutyrate synthase isolated from a host cell which expresses the synthase.

BSPR:
The invention further provides isolated and purified nucleic acid segments, e.g., in the form of an expression cassette, for each of the individual genes in the macrolide biosynthetic gene cluster. For example, the invention provides an isolated and purified pikAV gene that encodes a thioesterase II. In particular, the thioesterase is useful to enhance the structural diversity of antibiotics and in PHA production, as the thioesterase modulates chain release and cyclization. For example, a thioesterase II gene having acyl-ACP coenzyme A transferase activity (e.g., a mutant pik TEII, bacterial, fungal or plant medium-chain-length thioesterase, an animal fatty acid thioesterase or a thioesterase from a polyketide synthase) is introduced at the end of a recombinant monomer synthase (see FIG. 36), which, in the presence of a PHA synthase, e.g., phaC1, produces a novel polyhydroxyalkanoate polymer. Alternatively, in the absence of a TEII domain, a fusion of a portion of PKS gene cluster with a PHA synthase may result in the transfer of an acyl chain from the PHA to the polymerase.

BSPR:
Thus, the modules encoded by the nucleic acid segments of the invention may be employed in the methods described hereinabove to prepare polyhydroxyalkanoates of varied chain length or having various side chain substitutions and/or to prepare glycosylated biopolymers. Therefore, the compounds produced by the recombinant host cells of the invention are useful as biopolymers, e.g., in packaging or biomedical applications, or to engineer PHA monomer synthases; pharmaceuticals such as chemotherapeutic agents, immunosuppressants, agents to treat asthma, chronic obstructive pulmonary disease as well as other diseases involving respiratory inflammation, cholesterol-lowering agents, or macrolide-based antibiotics which are active against a variety of organisms, e.g., bacteria, including multi-drug-resistant pneumococci and other respiratory pathogens, as well as viral and parasitic pathogens; or as crop protection agents (e.g., fungicides or insecticides) via expression of polyketides in plants. Methods employing these compounds, e.g., to treat a mammal, bird or fish in need of such therapy, such as a patient having a bacterial infection, are also envisioned.

ORPL:
Poirier, Y., et al., "Production of Polyhydroxyalkanoates, a Family of Biodegradable Plastics and Elastomers, in Bacteria Plants", Bio/Technology, 13, 142-150 (1995).

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L2: Entry 3 of 98 File: USPT Jun 12, 2001

DOCUMENT-IDENTIFIER: US 6245537 B1
TITLE: Removing endotoxin with an oxdizing agent from polyhydroxyalkanoates produced by fermentation

ORPL:
Poirier, "Perspectives on the production of polyhydroxyalkanoates in plants," FEMS Microbiology Reviews 103: 237-246 (1992).

L2: Entry 5 of 98 File: USPT May 8, 2001

DOCUMENT-IDENTIFIER: US 6228623 B1
TITLE: Polyhydroxyalkanoates of narrow molecular weight distribution prepared in transgenic plants

BSPR:
The present invention relates to genetically engineered plants and bacteria. In particular, it relates to methods for optimizing substrate pools to facilitate the biosynthetic production of commercially useful levels of polyhydroxyalkanoates (PHAs) in bacteria and plants.

BSPR:
In another aspect, the present invention provides methods for the biological production of P(3HB-co-3HV) copolymers in plants and bacteria utilizing propionyl-CoA as a substrate. As shown in FIG. 3, propionyl-CoA can be produced through a variety of engineered metabolic pathways. Introduction into plants and bacteria of appropriate .beta.-ketothiolases capable of condensing acetyl-CoA with itself and/or with propionyl-CoA, along with appropriate .beta.-ketoacyl-CoA reductases and PHA synthases, in combination with various enzymes involved in asparate family amino acid biosynthesis and the conversion of threonine to PHA copolymer precursors, will permit these organisms to produce P(3HB-co-3HV) copolymers. The PHA biosynthetic enzymes can be those of A. eutrophus or other organisms, or enzymes that catalyze reactions involved in fatty acid biosynthesis or degradation, ultimately resulting in the conversion of acetyl-CoA and propionyl-CoA to P(3HB-co-3HV). In plants, these enzymes can be expressed in the cytoplasm or targeted to organelles such as plastids (e.g., those of leaves or seeds) or mitochondria via the use of transit peptides for enhanced production of polyhydroxyalkanoates. Alternatively, plastids can be transformed with recombinant constructs that facilitate expression of these enzymes directly within the plastids themselves.

BSPR:
Any of the foregoing plants, wherein said polyhydroxyalkanoate synthase is obtainable from a microorganism selected from the group consisting of Alcaligenes eutrophus, Alcaligenes faecalis, Aphanothece sp., Azotobacter vinelandii, Bacillus cereus, Bacillus megaterium, Beijerinkia indica, Derxia gummosa, Methylobacterium sp., Microcoleus sp., Nocardia corallina, Pseudomonas cepacia, Pseudomonas extorquens, Pseudomonas oleovorans, Rhodobacter sphaeroides, Rhodobacter capsulatus, Rhodospirillum rubrum, and Thiocapsa pfennigii.

BSPR:
A method of producing P(3HB-co-3HV)copolymer, comprising growing any of the foregoing plants, the genome of which comprises introduced DNAs encoding a .beta.-ketothiolase, a .beta.-ketoacyl-CoA reductase, and a polyhydroxyalkanoate synthase, and recovering said P(3HB-co-3HV) copolymer produced thereby.

BSPR:
In a preferred embodiment, a plant extract will contain a polyhydroxyalkanoate polymer wherein the polyhydroxyalkanoate polymer was produced by a plant, and where the polyhydroxyalkanoate polymer has a single mode molecular weight distribution. As used herein, a plant extract refers to materials prior to chromatographic separation. The extract may be a plant lysate, or the materials dissolved from contacting the plant or plant materials with a suitable solvent including, but not limited to alcohol or chloroform. Lysates may be prepared by a variety of methods including, but not limited to mechanical damage, chemical treatment, or enzymatic digestion. The polyhydroxyalkanoate polymer preferably has a molecular weight distribution of between about 1 and about 5, preferably

between about 1.5 and about 4.5, more preferably between about 2 and about 4, and most preferably about 2.1 or about 2.5. The polyhydroxyalkanoate polymer may be a homopolymer or a copolymer. The polyhydroxyalkanoate polymer may generally be any polyhydroxyalkanoate polymer compatible with the inventive processes, and more preferably a polymer of 3-hydroxybutyrate, 3-hydroxyhexanoate, 3-hydroxyoctanoate, 3-hydroxydecanoate, 3-hydroxydodecanoate, 3-hydroxytetradecanoate, 3-hydroxyhexadecanoate, 3-hydroxyoctadecanoate, 3-hydroxyeicosanoate, 3-hydroxydocosanoate, or copolymers thereof. In more preferred embodiments, the homopolymer is poly(3-hydroxybutyrate) or poly(4-hydroxybutyrate), and the to copolymer is poly(3-hydroxybutyrate-co-3-hydroxyvalerate), poly(3-hydroxybutyrate-co-4-hydroxybutyrate), poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), poly(4-hydroxybutyrate-co-3-hydroxyhexanoate), or poly(3-hydroxybutyrate-co-4-hydroxybutyrate-co-3-hydroxyhexanoate). The plant in which the polyhydroxyalkanoate polymer is biosynthesized is generally any plant suitable for the biosynthesis of polyhydroxyalkanoate polymers, and more preferably is tobacco, wheat, is potato, Arabidopsis, corn, soybean, canola, oil seed rape, sunflower, flax, peanut, sugarcane, swtichgrass, or alfalfa. The number average molecular weight of the polyhydroxyalkanoate polymer is preferably greater than about 100,000, more preferably greater than about 300,000, and most preferably greater than about 500,000.

BSPR:
The invention further provides methods for the preparation of polyhydroxyalkanoate polymers having a single mode molecular weight distribution. A preferred embodiment comprises the steps of (a) inserting into a plant cell nucleic acid molecules comprising a polyhydroxyalkanoate synthesis pathway, preferably comprising a .beta.-ketoacyl reductase gene, a .beta.-ketothiolase gene, and a polyhydroxyalkanoate synthase gene; (b) isolating a transformed plant cell; (c) regenerating the transformed plant cell to form a transformed plant; (d) selecting a transformed plant which produces a polyhydroxyalkanoate polymer having a single mode molecular weight distribution; and (e) isolating the polyhydroxyalkanoate polymer. The .beta.-ketoacyl reductase, .beta.-ketothiolase, and polyhydroxyalkanoate synthase genes may generally be of any source suitable for participation in the inventive processes, more preferably the genes are Alcaligenes eutrophus, Alcaligenes faecalis, Aphanothece sp., Azotobacter vinelandii, Bacillus cereus, Bacillus megaterium, Beijerinkia indica, Derxia gummosa, Methylobacterium sp., Microcoleus sp., Nocardia corallina, Pseudomonas cepacia, Pseudomonas extorquens, Pseudomonas oleovorans, Rhodobacter sphaeroides, Rhodobacter capsulatus, Rhodospirillum rubrum, or Thiocapsa pfennigii genes, and most preferably are Alcaligenes eutrophus genes. The polyhydroxyalkanoate polymer preferably has a molecular weight distribution of between about 1 and about 5, preferably between about 1.5 and about 4.5, more preferably between about 2 and about 4, and most preferably about 2.1 or about 2.5. The polyhydroxyalkanoate polymer may be a homopolymer or a copolymer. The polyhydroxyalkanoate polymer may generally be any polyhydroxyalkanoate polymer compatible with the inventive processes, and more preferably a polymer of 3-hydroxybutyrate, 3-hydroxyhexanoate, 3-hydroxyoctanoate, 3-hydroxydecanoate, 3-hydroxydodecanoate, 3-hydroxytetradecanoate, 3-hydroxyhexadecanoate, 3-hydroxyoctadecanoate, 3-hydroxyeicosanoate, 3-hydroxydocosanoate, or copolymers thereof. In more preferred embodiments, the homopolymer is poly(3-hydroxybutyrate) or poly(4-hydroxybutyrate), and the copolymer is poly(3-hydroxybutyrate-co-3-hydroxyvalerate), poly(3-hydroxybutyrate-co-4-hydroxybutyrate), poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), poly(4-hydroxybutyrate-co-3-hydroxyhexanoate), or poly(3-hydroxybutyrate-co-4-hydroxybutyrate-co-3-hydroxyhexanoate). The plant in which the polyhydroxyalkanoate polymer is biosynthesized is generally any plant suitable for the biosynthesis of polyhydroxyalkanoate polymers, and more preferably is tobacco, wheat, potato, Arabidopsis, corn, soybean, canola, oil seed rape, sunflower, flax, peanut, sugarcane, swtichgrass, or alfalfa. The number average molecular weight of the polyhydroxyalkanoate polymer is preferably greater than about 100,000, more preferably greater than about 300,000, and most preferably greater than about 500,000. The transformed plant cells and plants may further comprise nucleic acid molecules having genes that allow the plant to synthesize additional polyhydroxyalkanoate precursors. In a preferred embodiment, the nucleic acid molecules further comprise succinyl-CoA:acetyl-CoA CoA transferase, succinate semialdehyde dehydrogenase, 4-hydroxybutyrate

dehydrogenase, and hydroxybutyryl-CoA:acetyl-CoA CoA transferase genes. The inventive methods may be further extended by including a step of generating a homozygous daughter plant derived from the transformed plant. Conventional methods such as growing daughter plants from seeds, and cross-breeding may be used to generate such a homozygous daughter plant. The transformed plant may be analyzed to determine the copy number of the polyhydroxyalkanoate synthase gene. It is preferable that the synthase gene be present in a low copy number, more preferably less than five, and most preferably present at a single copy. Copy number may be determined by any method known to those of skill in the art, and preferably by Southern blotting.

BSPR:
The invention further provides methods for selecting a transformed plant cell or plant that is particularly suitable for the production of a polyhydroxyalkanoate polymer having a single mode molecular weight distribution. A preferred embodiment comprises the steps of (a) obtaining transformed plant cells or transformed plants, (b) analyzing the plant cells or plants for the presence of a polyhydroxyalkanoate synthase gene, (c) determining the copy number of the polyhydroxyalkanoate synthase gene, and (d) selecting a transformed plant cell or transformed plant having a single copy of the polyhydroxyalkanoate synthase gene. Copy number may be determined by any method known to those of skill in the art, and preferably by Southern blotting.

DEPR:
In view of the ability of heterologous bacterial and plant host cells to produce PHB polyhydroxyalkanoate upon introduction and expression therein of DNAs encoding the appropriate PHB biosynthetic enzymes, it is similarly expected that P(3HB-co-3HV) copolymer can be produced in bacteria and plants by expressing therein appropriate combinations of PHA-biosynthetic and other enzymes as discussed above. As the levels of acetyl-CoA required for copolymer biosynthesis in bacteria and plant cells appear to be non-limiting (Nawrath et. al., 1994), no further manipulation of cellular metabolism with respect to this precursor is likely to be necessary. Insuring the presence of sufficient pools of propionyl-CoA required for C4/C5 copolymer production may require introduction into, and overexpression in, host cells of various combinations of wild-type or deregulated aspartate kinase, homoserine dehydrogenase, threonine synthase, wild-type or deregulated threonine deaminase, .alpha.-ketoacid dehydrogenase E1, E2, and E3 subunits, pyruvate oxidase, and acyl-CoA synthetase enzymes.

DEPR:
Poly(4-hydroxybutyrate) and copolymers of 4-hydroxybutyrate and other hydroxyalkanoates such as 3-hydroxybutyrate, 3-hydroxyvalerate, and 3-hydroxyhexanoate may be biosynthesized in plants capable of producing 4-hydroxybutyryl-CoA. Polymers containing 4-hydroxybutyrate are desirable due to their improved material properties, e.g. increased tensile strength, when compared to other short chain polyhydroxyalkanoates. The substrate 4-hydroxybutyryl-CoA may be biosynthesized in plants by, for example, the Clostridium kluyveri pathway.

CLPR:
1. A plant extract comprising a polyhydroxyalkanoate polymer, wherein

CLPR:
2. The plant extract of claim 1, wherein the polyhydroxyalkanoate polymer has a molecular weight distribution of between about 1 and about 5.

CLPR:
3. The plant extract of claim 2, wherein the polyhydroxyalkanoate polymer has a molecular weight distribution of between about 2 and about 4.

CLPR:
4. The plant extract of claim 3, wherein the polyhydroxyalkanoate polymer has a molecular weight distribution of about 2.5.

CLPR:
5. The plant extract of claim 3, wherein the polyhydroxyalkanoate polymer has a molecular weight distribution of about 2.1.

CLPR:

6. The plant extract of claim 1, wherein the polyhydroxyalkanoate polymer is a polymer of 3-hydroxybutyrate, 3-hydroxyhexanoate, 3-hydroxyoctanoate, 3-hydroxydecanoate, 3-hydroxydodecanoate, 3-hydroxytetradecanoate, 3-hydroxyhexadecanoate, 3-hydroxyoctadecanoate, 3-hydroxyeicosanoate, 3-hydroxydocosanoate, or copolymers thereof.

CLPR:
7. The plant extract of claim 1, wherein the polyhydroxyalkanoate polymer is a homopolymer.

CLPR:
9. The plant extract of claim 1, wherein the polyhydroxyalkanoate polymer is a copolymer.

CLPR:
12. The plant extract of claim 1, wherein the polyhydroxyalkanoate polymer has a number average molecular weight greater than about 100,000.

CLPR:
13. The plant extract of claim 12, wherein the polyhydroxyalkanoate polymer has a number average molecular weight greater than about 300,000.

CLPR:
14. The plant extract of claim 13, wherein the polyhydroxyalkanoate polymer has a number average molecular weight greater than about 500,000.

CLPV:
the polyhydroxyalkanoate polymer was produced by the plant; and

CLPV:
(a) inserting into a plant cell nucleic acid molecules encoding a polyhydroxyalkanoate synthase pathway;

CLPV:
(d) selecting a transformed plant which produces a polyhydroxyalkanoate polymer having a single mode molecular weight distribution.

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L2: Entry 10 of 98 File: USPT Jan 16, 2001

DOCUMENT-IDENTIFIER: US 6175061 B1
TITLE: Production of polyhydroxyalkanoate in plants

ABPL:
A plant which produces polyhydroxy-alkanoate polymer has a recombinant genome which contains one or more than one of the genes specifying enzymes critical to the polyhydroxyalkanoate biosynthetic pathway which occurs in certain micro-organistas such as Alcaligenes eutrophus which naturally produce same. The plant species is preferably an oil-producing plant.

PCPR:
This invention relates to the production of polyhydroxyalkanoate in plants.

BSPR:
According to the present invention there is provided a plant adapted for the production of polyhydroxyalkanoate comprising a recombinant genome of an oil-producing plant, which genome contains genes encoding enzymes necessary for catalyzing the production of polyhydroxy-alkanoate together with gene regulatory sequences directing expression of the said genes to target plant call components.

BSPR:
An important preferred feature of this invention is the use of an oilseed plant for expression of the polyhydroxyalkanoate. The reason behind our selection of oil-producing crops is that such plants naturally produce large amounts of acetyl-CoA substrate (under aerobic conditions) in the developing seed, which is normally used in fatty acid synthesis. Diversion of this substrate into polyhydroxyalkanoate production will reduce the amount of oil stored by the seed but will have minimal influence on other aspects of the cell's metabolism. It is therefore possible to produce commercial viable quantities of polyhydroxy-alkanoate such as polyhydroxybutyrate in an oilseed.

BSPR:
For expression in higher plants the bacterial (for example Alcaligene eutrophus) genes require suitable promoter and terminator sequences. various promoters/torminators are available for use. For constitutive expression the cauliflower mosaic virus CaMV35S promoter and nos terminator may be used. It is however preferred to target synthesis of polyhydroxyalkanoate only to the developing oil storage organ of the oilseed such as the embryo of oilseed rape. The promoter of the rape seed storage protein, napin, could be used to obtain embryo specific expression of polyhydroxyalkanoate genes. Expression of the polyhydroxyalkanoate genes during the precise period when lipid is being made will ensure effective omrpetition by the polyhydroxyalkanoate enzymes for available acetyl-CoA. The promoters of fatty acid synthesis genes whose expressions are switched on at this time are thus most appropriate candidates to be used as polyhydroxyalkanoate gene promoters. Examples of such promoters are those of seed specific isoforms of rape acyl carrier protein (ACP) or .beta.-ketoacyl ACP reductase.

BSPR:
To target the three bacterial (such as Alcaligenes eutrophus) enzymes for polyhydroxyalkanoate synthesis to the plant plastid requires the use of specific targeting regulatory elements called transit peptides. Possible sources of plastid stroma targeting sequences are the genes for:

BSPR:

Of these the RUBISCO small subunit transit peptide has been shown to direct polypeptides to plastids in both photosynthetic and non-photosynthetic tissues. ACP and .beta.-ketoacyl ACP reductase transit peptides would also operate effectively in plants such as rape embryo. The advantage of using the same plastid transit peptide for all three polyhydroxyalkanoate genes is to ensure that any variability in the uptake of the genes is not due to the transit peptide which is used.

BSPR:
To obtain synthesis of polyhydroxyalkanoate polymer in plant tissues it is necessary to obtain plants expressing all three genes for the enzymes .beta.-ketothiolass, acetoacetyl-CoA reductase and polyhydroxybutyrate synthase. This may be achieved by using one of the following strategies:

BSPL:
A combination of these techniques may be used to obtain expression of all three genes in a single plant. successive round of cross-pollination are carried out until the progeny are homozygous for all three genes. For methods (ii) and (iii) above, it is advantageous to insert each gene into vectors containing different selectable marker genes to facilitate selection of plants containing two or three polyhydroxyalkanoate pathway genes. Examples of selectable markers are genes conferring resistances to kanamycin, hygromycin, sulphonamides and bialaphos or phosphinothricin.

BSPV:
i) Plants may be individually transformed with the three polyhydroxyalkanoate pathway genes. Plants containing individual genes are grown up in the glass-house and cross-pollinated to obtain hybrid plants containing two pathway genes. This procedure is then repeated to produce hybrid plants containing all three genes.

CLPR:
1. An oil-producing plant selected from the group consisting of oilseed rape, canola, soya, sunflower, and tobacco that produces polyhydroxyalkanoate, the plant comprising a recombinant genome, wherein the genome contains:

CLPR:
2. The plant of claim 1, wherein the genes encoding .beta.-ketothiolase, acetoacetyl-CoA reductase, and polyhydroxyalkanoate synthase proteins are isolated from a micro-organism.

CLPR:
4. The plant of claim 1, in which the polyhydroxyalkanoate synthase is a polyhydroxybutyrate synthase.

CLPR:
5. A Brassica plant that produces polyhydroxyalkanoate, the plant comprising a recombinant genome, wherein the genome contains:

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L2: Entry 17 of 98 File: USPT Sep 12, 2000

DOCUMENT-IDENTIFIER: US 6117658 A
TITLE: Methods of making polyhydroxyalkanoates comprising 4-hydroxybutyrate monomer units

BSPR:
The field of PHA production has altered its focus in recent years to include plant produced PHAs (Eschenlauer, A. C. et al., Abstr. Annul. Meet. International Symposium on Bacterial Polyhydroxyalkanoates, Montreal, Canada, 85:66, 1994; Hahn, J. J., Abstr. Annu. Meet. International Symposium on Bacterial Polyhydroxyalkanoates, Montreal, Canada, 82:65; Landschulze, V. et al., Abstr. Annu. Meet. International Symposium on Bacterial PHA, Montreal, Canada, 86:66; Nawrath, C. et al., Abstr. Annu. Meet. International Symposium on Bacterial Polyhydroxyalkanoates, Montreal, Canada, 83, 1994; Nawrath, C., Proc. Natl. Acad. Sci. 91:12760-64, 1994; Poirier, Y., Adv. Mater. 5:30-36, 1993; Pool, R., Science 245:1187-1189, 1989; Poirier, Y., Sci. 256:520-523, 1992). Such technology includes the expression of the A. eutrophus pha synthesis genes in Arabidopsis thaliana (Poirier, Y., Sci. 256:520-523, 1992) with the formation of PHA reaching 14% of the dry weight of the leaves (Nawrath, C., et al., Proc. Nat. Acad. Sci. USA 91:12760-12764). PHA production in plants may be less expensive than PHA production in bacteria.

BSPR:
The present invention provides preferred sources and host cells, which are discussed in further detail in the Detailed Description of the Invention, below, and that include the following. The succinic semialdehyde metabolic pathway can be from Clostridium kluyveri, while the polyhydroxyalkanoate biosynthetic pathway can be from Ralstonia eutropha and the polyhydroxyalkanoate synthase from Nocardia corallina. Preferrred host cells include Escherichia coli, Klebsiella, particularly Klebsiella aerogenes and Klebsiella oxytoca, plant host cells, insect host cells and spider host cells. In one preferred embodiment, the host cell has an inhibiting mutation in its CoA-independent NAD-dependent and/or NADP-dependent succinic semialdehyde dehydrogenase.

BSPR:
In yet another aspect, the present invention provides a transgenic plant whose germ or somatic cells contain at least one recombinant sequence that encodes a polyhydroxyalkanoate biosynthetic pathway, and at least one sequence that encodes a succinic semialdehyde metabolic pathway that metabolizes succinic semialdehyde via a 4-hydroxybutyryl-CoA intermediate. In a preferred embodiment, the plant is Arabidopsis thaliana. In another preferred embodiment, the polyhydroxyalkanoate biosynthetic pathway comprises a polyhydroxyalkanoate synthase from Nocardia corallina pha operably linked to a promoter such that the gene is able to be expressed in the transgenic plant.

DEPR:
An example of a plant suitable for use within the present invention is Arabidopsis thaliana. (Nawrath, C., Proc. Natl. Acad. Sci. 91:12960-64, 1994; Poirier, Y., et al., Science 256:520-523, 1992). The use of plant cells can be particularly advantageous because the PHA biosynthetic pathway can utilize the fatty acid degradation or fatty acid biosynthesis pathways in plants, which in some plants results in the accumulation of fatty acids in the form of oils to levels as high as 30 to 40% of their weight (Ohlrogge, J. B., Jaworski, J. G. and Post-Beittenmiller, Lipid Metabolism In Plants, 1:3-33 (CRC Press, Moore, T. S., ed., 1993). (See also Eschenlauer, A. C. et al., Abstr. Annu. Meet. International Symposium on Bacterial Polyhydroxyalkanoates, Montreal, Canada, 85:66, 1994; Hahn, J. J., Abstr. Annu. Meet. International Symposium on Bacterial Polyhydroxyalkanoates, Montreal, Canada, 82:65; Landschulze, V. et al., Abstr.

Polyhydroxyalkanoates, Montreal, Canada, 82:65; Landschulze, V. et al., Abstr. Annu. Meet. International Symposium on Bacterial PHA, Montreal, Canada, 86:66; Nawrath, C. et al., Abstr. Annu. Meet. International Symposium on Bacterial Polyhydroxyalkanoates, Montreal, Canada, 83, 1994; Poirier, Y., Adv. Mater. 5:30-36, 1993; Pool, R., Science 245:1187-1189, 1989; Poirier, Y., Sci. 256:520-523, 1992; Nawrath, C., Proc. Natl. Acad. Sci. 91:12760-64, 1994).

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Search Results - Record(s) 11 through 20 of 98 returned.

☐ 11. Document ID: US 6150586 A

L2: Entry 11 of 98 File: USPT Nov 21, 2000

US-PAT-NO: 6150586
DOCUMENT-IDENTIFIER: US 6150586 A

TITLE: Plant gene encoding acetyl coenzyme a carboxylase biotin carboxyl carrier protein

DATE-ISSUED: November 21, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Slabas; Antoni Ryszard	Durham	N/A	N/A	GBX
Elborough; Kieran Michael	Cleveland	N/A	N/A	GBX

US-CL-CURRENT: 800/281; 435/419, 435/468, 536/23.6, 800/286, 800/298

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

☐ 12. Document ID: US 6143952 A

L2: Entry 12 of 98 File: USPT Nov 7, 2000

US-PAT-NO: 6143952
DOCUMENT-IDENTIFIER: US 6143952 A

TITLE: Modified pseudomonas oleovorans phaC1 nucleic acids encoding bispecific polyhydroxyalkanoate polymerase

DATE-ISSUED: November 7, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Srienc; Friedrich	Lake Elmo	MN	N/A	N/A
Jackson; John K.	Plymouth	MN	N/A	N/A
Somers; David A.	Roseville	MN	N/A	N/A

US-CL-CURRENT: 800/298; 435/252.3, 435/254.2, 435/320.1, 435/440, 435/468, 435/471, 435/69.1, 536/23.2, 536/23.7, 800/278

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

☐ 13. Document ID: US 6143561 A

L2: Entry 13 of 98 File: USPT Nov 7, 2000

US-PAT-NO: 6143561
DOCUMENT-IDENTIFIER: US 6143561 A

TITLE: DNA encoding plastid pyruvate dehydrogenase and branched chain oxoacid dehydrogenase components

DATE-ISSUED: November 7, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Randall; Douglas D.	Columbia	MO	N/A	N/A
Mooney; Brian P.	Columbia	MO	N/A	N/A
Johnston; Mark L.	Gales Ferry	CT	N/A	N/A
Luethy; Michael H.	Old Mystic	CT	N/A	N/A
Miernyk; Jan A.	Peoria	IL	N/A	N/A

US-CL-CURRENT: 435/419; 435/252.3, 435/320.1, 536/23.2, 536/23.6

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

☐ 14. Document ID: US 6127606 A

L2: Entry 14 of 98 File: USPT Oct 3, 2000

US-PAT-NO: 6127606
DOCUMENT-IDENTIFIER: US 6127606 A

TITLE: Method of using transactivation proteins to control expression in transgenic plants

DATE-ISSUED: October 3, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bennett; Malcolm	Coventry	N/A	N/A	GBX
May; Sean	Earlsdon	N/A	N/A	GBX
Ramsay; Nicola	Bishopston	N/A	N/A	GBX

US-CL-CURRENT: 800/298; 435/320.1, 435/419, 435/468, 536/23.6, 536/23.7, 536/24.1, 800/278, 800/295

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

☐ 15. Document ID: US 6127512 A

L2: Entry 15 of 98 File: USPT Oct 3, 2000

US-PAT-NO: 6127512
DOCUMENT-IDENTIFIER: US 6127512 A

TITLE: Plasticized polyhydroxyalkanoate compositions and methods for their use in the production of shaped polymeric articles

DATE-ISSUED: October 3, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Asrar; Jawed	Chesterfield	MO	N/A	N/A
Pierre; Jean R.	St-Denis	N/A	N/A	BEX

US-CL-CURRENT: 528/272; 528/271

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw Desc | Image

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☐ 16. Document ID: US 6127166 A

L2: Entry 16 of 98 File: USPT Oct 3, 2000

US-PAT-NO: 6127166
DOCUMENT-IDENTIFIER: US 6127166 A

TITLE: Molluscan ligament polypeptides and genes encoding them

DATE-ISSUED: October 3, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bayley; Hagan	College Station	TX	77845	N/A
Cao; Qiuping	Shrewsbury	MA	01545	N/A
Wang; Yunjuan	Bryan	TX	77801	N/A

US-CL-CURRENT: 435/252.3; 435/320.1, 435/325, 435/69.1, 536/23.1, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw Desc | Image

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☐ 17. Document ID: US 6117658 A

L2: Entry 17 of 98 File: USPT Sep 12, 2000

US-PAT-NO: 6117658
DOCUMENT-IDENTIFIER: US 6117658 A

TITLE: Methods of making polyhydroxyalkanoates comprising 4-hydroxybutyrate monomer units

DATE-ISSUED: September 12, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dennis; Douglas E.	Weyers Cave	VA	N/A	N/A
Valentin; Henry E.	Chesterfield	MO	N/A	N/A

US-CL-CURRENT: 435/135; 435/141, 435/146, 435/170

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw Desc | Image

☐ 18. Document ID: US 6103956 A

L2: Entry 18 of 98 File: USPT Aug 15, 2000

US-PAT-NO: 6103956
DOCUMENT-IDENTIFIER: US 6103956 A

TITLE: Polyhydroxyalkanoate synthesis in plants

DATE-ISSUED: August 15, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Srienc; Friedrich	Lake Elmo	MN	N/A	N/A
Somers; David A.	Roseville	MN	N/A	N/A
Hahn; J. J.	New Brighton	MN	N/A	N/A
Eschenlauer; Arthur C.	Circle Pines	MN	N/A	N/A

US-CL-CURRENT: 800/298; 435/135, 435/419, 800/278

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw Desc | Image

☐ 19. Document ID: US 6096810 A

L2: Entry 19 of 98 File: USPT Aug 1, 2000

US-PAT-NO: 6096810
DOCUMENT-IDENTIFIER: US 6096810 A

TITLE: Modified polyhydroxyalkanoates for production of coatings and films

DATE-ISSUED: August 1, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Asrar; Jawed	Chesterfield	MO	N/A	N/A
D'Haene; Pol	Kessel-Lo	N/A	N/A	BEX

US-CL-CURRENT: 524/80; 560/127

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw Desc | Image

☐ 20. Document ID: US 6091002 A

L2: Entry 20 of 98 File: USPT Jul 18, 2000

US-PAT-NO: 6091002
DOCUMENT-IDENTIFIER: US 6091002 A

TITLE: Polyhydroxyalkanoates of narrow molecular weight distribution prepared in transgenic plants

DATE-ISSUED: July 18, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Asrar; Jawed	Chesterfield	MO	N/A	N/A
Mitsky; Timothy A.	Maryland Heights	MO	N/A	N/A
Shah; Devang T.	Chesterfield	MO	N/A	N/A

US-CL-CURRENT: 800/288; 435/135, 800/260

Full | Title | Citation | Front | Review | Classification | Date | Reference

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Term	Documents
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PLANT.DWPI,EPAB,JPAB,USPT,PGPB.	403781
PLANTA.DWPI,EPAB,JPAB,USPT,PGPB.	2057
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PLANTAG.DWPI,EPAB,JPAB,USPT,PGPB.	2
PLANTAL.DWPI,EPAB,JPAB,USPT,PGPB.	5
PLANTAN.DWPI,EPAB,JPAB,USPT,PGPB.	127
PLANTAR.DWPI,EPAB,JPAB,USPT,PGPB.	2962
PLANTAS.DWPI,EPAB,JPAB,USPT,PGPB.	49
(PLANT$2 SAME POLYHYDROXYALKANOATE$2).USPT,PGPB,JPAB,EPAB,DWPI.	98

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☐ 1. Document ID: US 6143952 A

L5: Entry 1 of 2 File: USPT Nov 7, 2000

US-PAT-NO: 6143952
DOCUMENT-IDENTIFIER: US 6143952 A
TITLE: Modified pseudomonas oleovorans phaC1 nucleic acids encoding bispecific polyhydroxyalkanoate polymerase

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 6103956 A

L5: Entry 2 of 2 File: USPT Aug 15, 2000

US-PAT-NO: 6103956
DOCUMENT-IDENTIFIER: US 6103956 A
TITLE: Polyhydroxyalkanoate synthesis in plants

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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PLANT.USPT.	149288
PLANTA.USPT.	1964
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PLANTAL.USPT.	3
PLANTAN.USPT.	76
PLANTAR.USPT.	2123
PLANTAS.USPT.	45
PLANTAT.USPT.	1
(PLANT$2 SAME POLYHYDROXYALKANOATE$2 SAME PEROXISOM$2).USPT.	2

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PLANTAN.USPT.	76
PLANTAR.USPT.	2123
PLANTAS.USPT.	45
PLANTAT.USPT.	1
(PLANT$2 SAME POLYHYDROXYALKANOATE$2 SAME PEROXISOM$2).USPT.	2

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USPT,PGPB,JPAB,EPAB,DWPI	plant$2 same polyhydroxyalkanoate$2	98	L2
USPT,PGPB,JPAB,EPAB,DWPI	plant same polyhydroxyalkanoate$2	98	L1

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L2: Entry 18 of 98 File: USPT Aug 15, 2000

DOCUMENT-IDENTIFIER: US 6103956 A
TITLE: Polyhydroxyalkanoate synthesis in plants

ABPL:
Novel transgenic plants and plant cells are capable of biosynthesis of polyhydroxyalkanoate (PHA). Heterologous enzymes involved in PHA biosynthesis, particularly PHA polymerase, are targeted to the peroxisome of a transgenic plant. Transgenic plant materials that biosynthesize short chain length monomer PHAs in the absence of heterologous .beta.-ketothiolase and acetoacetyl-CoA reductase are also disclosed.

BSPR:
The present invention provides a system for producing polyhydroxyalkanoate (PHA) in transgenic plant cells. Preferably, PHA is synthesized in differentiated plant cells, more preferably, in whole plants. Biopolymers comprising biologically synthesized PHA can be isolated from one or more differentiated cells, tissues, organs, or other components of a transgenic plant, including leaves, stems, seeds, fruits, buds, tubers and roots. Formation of PHA in the transgenic plant material occurs by way of polymerization of one or more hydroxyalkanoates and is catalyzed by a heterologous polyhydroxyalkanoate (PHA) polymerase.

BSPR:
PHA.sub.SCL polymerase, preferably a peroxisomally targeted PHA.sub.SCL polymerase, wherein expression of the heterologous nucleotide sequence encoding the PHA.sub.SCL polymerase in the transgenic plant cell in the absence of functional heterologous .beta.-ketothiolase and functional heterologous acetoacetyl-CoA reductase leads to production of polyhydroxyalkanoate. Transgenic plants comprising the differentiated plant cell are also included in the invention.

BSPR:
Methods for making transgenic plants in accordance with the invention are also provided. In one embodiment, a plant cell is transformed with a nucleic acid fragment comprising a heterologous nucleotide sequence encoding a peroxisomally-targeted polyhydroxyalkanoate (PHA) polymerase to yield a transgenic plant cell, and the transgenic plant cell is grown into a transgenic plant. Preferably, expression of the heterologous nucleotide sequence encoding the peroxisomally-targeted PHA polymerase leads to the production in the transgenic plant of PHA. In a particularly preferred embodiment, the peroxisomally-targeted PHA polymerase is a peroxisomally-targeted PHA.sub.SCL polymerase, and expression of the heterologous nucleotide sequence encoding it leads to production of PHA even in the absence of functional heterologous .beta.-ketothiolase and functional heterologous acetoacetyl-CoA reductase. In another embodiment, the peroxisomally-targeted PHA polymerase is a peroxisomally-targeted PHA.sub.MCL polymerase.

BSPR:
The invention further provides a method for making PHA comprising transforming a plant cell with a nucleic acid fragment comprising a heterologous nucleotide sequence encoding a peroxisomally-targeted PHA polymerase; expressing the heterologous nucleotide sequence in the resulting transgenic plant cell to cause production of polyhydroxyalkanoate; then isolating and, optionally, purifying the resulting polyhydroxyalkanoate. Preferably, PHA is made in a transgenic plant of the invention, and is isolated from at least one tissue or organ of the transgenic plant, such as seed, a root, a stem, a tuber, a leaf, a fruit and a bud; more preferably, PHA is isolated from a seed, most preferably, a germinating

seed. The invention includes PHA-containing transgenic plant tissues, organs, and components, including seeds and germinating seeds, that are generated in accordance with the invention.

DEPR:
The present invention relates to the expression of heterologous genes involved in the synthesis pathway of polyhydroxyalkanoate biopolymers in transgenic plant cells. A "heterologous" nucleic acid fragment, or gene, is one containing a nucleotide sequence that is not normally present in the cell, for example a procaryotic nucleotide sequence that is present in a eucaryotic cell. A heterologous gene is also referred to herein as a transgene. As used herein, "transgenic" refers to an organism in which a nucleic acid fragment containing a heterologous nucleotide sequence has been introduced. The transgenes in the transgenic organism are preferably stable and inheritable. The heterologous nucleic acid fragment may or may not be integrated into the host genome.

DEPR:
The present invention allows for a "single gene" type transformation of a plant cell with a heterologous nucleotide sequence encoding a PHA.sub.SCL polymerase. That is, a nucleotide sequence encoding a heterologous PHA.sub.SCL polymerase is introduced into a plant cell without co-transforming the plant cell with nucleotide sequences encoding either of a heterologous .beta.-ketothiolase or a heterologous acetoacetyl-CoA reductase. In a "single gene" type transformation, the expression of a nucleotide sequence encoding the PHA.sub.SCL polymerase leads to production of polyhydroxyalkanoate in the transgenic cell in the absence of functional heterologous .beta.-ketothiolase or a functional heterologous acetoacetyl-CoA reductase. Preferably, the plant cell is a differentiated plant cell. The "single gene" used to transform the plant cell is preferably PHB polymerase, more preferably phbC from R. eutropha.

DEPR:
In particular, each of the PHA synthesis pathway genes was fused to constitutive transcription and translation initiation and termination signals derived from the cauliflower mosaic virus 35S promoter (CaMV 35S) and the nopaline synthase terminator from the Agrobacterium tumefaciens Ti plasmid (nos ter). Each construct was modified for transformation of BMS from one of three constructs, pBI-THIO, pBI-RED, and pBI-POL, as described by Poirier et al to produce PHB in Arabidopsis thaliana. See, e.g., "Polyhydroxybutyrate, a Biodegradable Thermoplastic, Produced in Transgenic Plants," Science, 256:520-522, 1992, and "Production of Polyhydroxyalkanoates, A Family of Biodegradable Plastics and Elastomers, in Bacteria and Plants," Bio/Technology, 13:142-150, 1992. Each gene, together with the CaMV 35S and nos ter sequences, was removed from the pBI 121 backbone (a low-copy plasmid for Agrobacterium-mediated transformation) with Hind III and EcoR I restriction endonucleases and transferred to a pUC119 backbone, a high-copy plasmid from which a great quantity of DNA may be prepared as required by the microprojectile bombardment procedure. Transformants were selected on MS2D medium with 3 .mu.g/mL of the herbicide phosphinothricin (PPT).

DEPR:
Obtaining PHB synthesis with a single enzyme in the peroxisome is surprising to a certain extent because the D-hydroxyacyl-CoAs required for PHA synthesis are the products of only a special case of fatty acid degradation (Kindl, "Fatty Acid Degradation in Plant Peroxisomes: Function and Biosynthesis of the Enzymes Involved," Biochemie, 75:225-230, 1993). Furthermore, only fatty acyl-CoAs with a cis double bond between the second and third highest numbered carbon atoms will produce a D-hydroxybutyryl-CoA, which is necessary for biosynthesis of PHB, and none of the common plant lipids contain a double bond at this position (Topfer et al., "Modification of Plant Lipid Synthesis," Science, 268:681-686, 1995). It is also worth noting that the PHA.sub.SCL polymerase enzyme alone was not sufficient to induce PHB biosynthesis in E. coli(Gerngross et al., "Overexpression and Purification of the Soluble Polyhydroxyalkanoate Synthase from Alcaligenes eutrophus: Evidence for a

CLPR:
1. A transgenic plant comprising recombinant DNA comprising a heterologous nucleotide sequence encoding a polyhydroxyalkanoate (PHA) polymerase operably linked to a peroxisome-targeting sequence.

CLPR:

9. The transgenic plant of claim 1 which produces polyhydroxyalkanoate.

CLPR:
10. A transgenic plant comprising recombinant DNA comprising a heterologous nucleotide sequence encoding a polyhydroxyalkanoate (PHA) polymerase operably linked to a peroxisome-targeting sequence, wherein expression of the heterologous nucleotide sequence leads to the production of polyhydroxyalkanoate in the plant.

CLPR:
12. The transgenic plant of claim 11 wherein the polyhydroxyalkanoate is produced in a plant tissue selected from the group consisting of a seed, a root, a stem, a tuber, a rhizome, a leaf, a fruit and a bud.

CLPR:
15. The transgenic plant of claim 10 wherein the polyhydroxyalkanoate is produced in a plant tissue selected from the group consisting of a seed, a root, a stem, a tuber, a rhizome, a leaf, a fruit and a bud.

CLPR:
16. A transgenic differentiated plant cell comprising recombinant DNA comprising a heterologous nucleotide sequence encoding a PHA.sub.SCL polymerase operably linked to a peroxisome-targeting sequence, wherein expression of the heterologous nucleotide sequence encoding the PHA.sub.SCL polymerase in the transgenic plant cell in the absence of functional heterologous .beta.-kethothiolase and functional heterologous acetoacetyl-CoA reductase leads to production of polyhydroxyalkanoate.

CLPR:
18. A transgenic differentiated plant cell comprising recombinant DNA comprising a heterologous nucleotide sequence encoding a polyhydroxyalkanoate (PHA) polymerase operably linked to a peroxisome-targeting sequence.

CLPR:
25. The method of claim 24 wherein expression of the heterologous nucleotide sequence encoding the PHA polymerase leads to the production of polyhydroxyalkanoate in the transgenic plant.

CLPR:
26. The method of claim 24 wherein the PHA polymerase comprises a PHA.sub.SCL polymerase, and wherein expression of the heterologous nucleotide sequence encoding the PHA.sub.SCL polymerase in the transgenic plant cell in the absence of functional heterologous .beta.-ketothiolase and functional heterologous acetoacetyl-CoA reductase leads to production of polyhydroxyalkanoate.

CLPR:
29. A method for making polyhydroxyalkanoate comprising isolating polyhydroxyalkanoate from at least one tissue of the transgenic plant of claim 10.

CLPV:
(a) transforming a plant cell with recombinant DNA comprising a heterologous nucleotide sequence encoding a polyhydroxyalkanoate (PHA) polymerase operably linked to a peroxisome-targeting sequence, to yield a transgenic plant cell; and

CLPV:
(a) transforming a plant cell with recombinant DNA comprising a heterologous nucleotide sequence encoding a polyhydroxyalkanoate (PHA) polymerase operably linked to a peroxisome-targeting sequence to yield a transgenic plant cell, wherein expression of the heterologous nucleotide sequence encoding the PHA polymerase in the transgenic plant cell leads to production of polyhydroxyalkanoate; and